Effects of N:P Ratio on the Occurrence of Harmful Algal Blooms

Honors Thesis

James Palus

Urban and agricultural development has had dramatic effects on the biogeochemical cycles of nitrogen (N) and phosphorus (P). Such nutrient-rich conditions cause cyanobacteria to dominate the phytoplankton, thereby causing a number of problems in the water bodies they inhabit. Cyanobacteria exhibit rapid growth under high nutrient conditions, and as such have been increasingly referred to as harmful algal blooms (HABs) when they become overabundant in aquatic ecosystems. While nutrient run-off is thought to be the primary cause of HABs, it has been found that it is not necessarily the independent quantities of nitrogen and phosphorus that favor cyanobacteria, but rather the N:P ratio. Interestingly, management strategies that aim to increase N:P ratios in lakes have not always resulted in the dominance of less harmful phytoplankton. Choctaw Lake, located in Madison County, OH, has been experiencing HABs that have been producing high levels of cyanotoxins. This study examined how the N:P ratio influences phytoplankton assemblage and dominance of cyanobacteria in lakes. As expected, there was a strong correlation ($r^2 = 0.91$) between increasing cyanobacteria dominance and low N:P ratios. A similarly strong relationship ($r^2 = 0.89$) was found between total algal biomass and Secchi depth, providing a potentially effective and low-cost monitoring tool for lake managers. However, caution should be taken when extrapolating this relationship to other systems with potentially different algae communities. Further research should investigate the nutrient constituents of the lake sediments to understand how they may be contributing to cyanobacteria dominance in the lake.

Introduction

Urban and agricultural development has had dramatic effects on the biogeochemical cycles of nitrogen (N) and phosphorus (P) (Vitousek et al. 1997). This development has resulted in non-point source pollution, the origins of which are difficult to trace. Sources include runoff from agriculture, urban settings, construction sites, septic tank leachate, and logging, among others (Carpenter et al. 1998). Further, industrial activity has increased atmospheric concentrations of nitrogen, doubling the amount of fixed nitrogen (Vitousek et al. 1997). The additive effects of each of these sources make for more difficult regulation than point-source pollution (Carpenter et al. 1998). The removal or degradation of riparian areas, whether forested or simply grass, has further exacerbated the issue, allowing nutrients easier access to waterways (Osborne and Kovacic 1993). These effects are most prevalent in aquatic ecosystems—particularly lakes—where eutrophication is often observed because of the increased load of nitrogen and phosphorus. Such nutrient-rich conditions cause cyanobacteria (i.e. blue-green algae) to dominate the phytoplankton, which cause a number of problems in the water bodies they inhabit, including toxic algae blooms and mass fish kills (MacKintosh 1990; Jochimsen et al. 1998; Hillerbrand et al. 2008; Vrede et al. 2009; Pinto et al. 2010).

When cyanobacteria exhibit rapid growth under high nutrient conditions, they frequently produce a harmful algal bloom (HAB). Such HABs have been shown to have several different negative impacts on aquatic ecosystems. For example, several species of cyanobacteria (e.g. species in the genera *Microcystis*, *Oscillatoria*, and *Anabaena*) release toxins, which can, in extreme cases, lead to liver failure in humans who come in contact with the water (Carmichael et al. 1985; Codd and Poon 1988; Rouhilainen et al. 1995; Jochimsen et al. 1998; Codd et al. 1999). In addition, these cyanotoxins have been responsible for the deaths of many aquatic and

terrestrial animals that have come in contact with contaminated water (Codd et al. 1989, Falconer 1989). HABs can also negatively impact aquatic ecosystems when the biomass accumulated during these blooms quickly dies and sinks to the sediment where it is decomposed. This decomposition process consumes dissolved oxygen, and in turn creates anoxic conditions, leading to "dead zones" (Trimbee and Prepas 1988) that can cause widespread fish kills. Finally, other problems commonly associated with cyanobacterial blooms include unappealing aesthetics, foul odor, and the clogging of feeding apparatuses of zooplankton (Webster and Peters 1978).

While nutrient run-off is thought to be the primary cause of HABs, interestingly, it has been found that it is not necessarily the independent quantities of nitrogen and phosphorus that favor cyanobacteria, but rather the ratio of nitrogen to phosphorus (N:P ratio) (Ferber et al. 2004; Vrede et al. 2009). In particular, cyanobacteria tend to dominate when there is a low N:P ratio, typically below 16:1 (Pearsall 1932; Redfield 1936; Schindler 1977; Smith 1983; Kahru et al. 2000). This is because cyanobacteria are at a competitive advantage over other algae because of their unique ability to either fix atmospheric nitrogen (N_2) or exploit sedimentary sources of ammonium (NH_4^+) for growth and reproduction (Ferber et al. 2004). In other words, when there is little nitrogen available in the water column relative to phosphorus, cyanobacteria can still thrive on sources of nitrogen unavailable to other phytoplankton. Cyanobacteria are able to use sedimentary sources of nitrogen because they are equipped with vacuoles that aid in the control of their buoyancy, allowing them to efficiently sink to the substrate and float back to the surface. In addition, cyanobacteria are capable of taking up dissolved inorganic phosphorus (DIP), which further leads to their ability to outcompete other phytoplankton species (Huber and Hamel 1985; Mulholland et al. 2002). It may therefore seem intuitive to suggest offering green algae a competitive advantage by increasing the N:P ratio in affected ecosystems.

Increasing the N:P ratio by decreasing P inputs poses several challenges, and so manipulating the ratio by instead increasing N inputs may provide an alternative solution. Interestingly, management strategies that aim to increase N and hence increase N:P ratios in lakes, have not always resulted in control of cyanobacteria by enhancing the competitive advantage of green algae and diatoms (i.e., non-toxic phytoplankton) (Barica et al. 1980; Stockner and Shortweed 1988). For example, Barica et al. (1980) manipulated N:P ratios in experimental lakes in Manitoba, Canada, adding nitrogen in either small or large amounts. While large additions (7–14 $g \cdot m^{-3} \cdot wk^{-1}$) changed the algal composition from cyanobacteria to chlorophytes, smaller additions (0.75–1.5 $g \cdot m^{-3} \cdot wk^{-1}$) simply resulted in a change from heterocystous cyanobacteria to non-heterocystous cyanobacteria (Barica et al. 1980). The large additions were successful in eliminating the toxic cyanobacteria, but still resulted in fish kills. Rather than oxygen depletion through decomposition, these fish kills are likely a result of diel fluctuations from algal respiration at night. However, the small additions merely eliminated one harmful cyanobacterium in favor of another.

Achieving successful results from manipulating the N:P ratio may prove difficult because of physiological differences among cyanobacteria. Specifically, the relative dominance of one species of cyanobacteria over another might be due to variation in the ability to fix nitrogen. This variation arises because not all species of cyanobacteria contain heterocystous cells, which are the sites of nitrogen fixation (Ferber et al. 2004). Therefore, low N:P ratios might lead to the dominance of not just cyanobacteria in general, but of heterocystous species of cyanobacteria, in particular, like those found in the Manitoba lakes by Barica et al. (1980). This study aims to test the hypothesis that low N:P ratios favor heterocystous species of cyanobacteria over non-heterocystous species.

Many advances have been made in the technology used to monitor HABs and detect the presence of algal toxins, such as remote sensing and handheld fluorometers (Marion et al. 2012). However, these are frequently not accessible to private, community-managed lakes (i.e., as opposed to lakes managed by state and federal agencies). Instead, private lake managers frequently rely on citizen-science monitoring programs to track HABs. For example, the Citizen Lake Awareness and Monitoring program (CLAM), supported by the Ohio Lake Management Society (OLMS), trains community-based managers to measure water temperature, secchi-disk depth, and water color (OLMS Website). As secchi depth measures water clarity, it has been used as an indirect measure of algal biomass (Sellner et al. 2003), i.e., higher turbidity/lower water clarity is assumed to be due to high phytoplankton abundance in the water column. While secchi depth is capable of providing rough estimates of algal biomass, it is incapable of discriminating between relatively benign green algae and harmful cyanobacteria (Nurnberg and Shaw 1999, Havens et al. 2003). As such, it is criticized as not being an effective tool for monitoring the occurrence of HABs (Sellner et al. 2003). Yet few studies have compared secchi depth measurements with direct measurements that quantify the biomass of cyanobacteria. One of the goals of this study is to assess the utility of secchi depth measurements in serving as an indirect measurement of the occurrence of HABs.

Choctaw Lake is a private lake located in Madison County, OH, that has been experiencing HABs that produce high levels of cyanotoxins. Interestingly, although heterocystous species, such as *Anabaena* sp., are present in the lake, HABs in Choctaw Lake have been dominated by *Planktothrix* spp., a non-heterocystous cyanobacterium capable of producing the hepatotoxin microcystin. As the lake is used primarily for recreation, these blooms prevent the local residents from being able to enjoy the lake, as the toxin levels pose actual

dangers to human health. Understanding more about these cyanobacteria and the processes involved in creating HABs will help the managers of the lake decide what actions to take in order to improve recreational safety in addition to downstream water quality.

This study aims to: 1) understand the relationship between nutrients and the dominance of cyanobacteria, and 2) evaluate how species traits (i.e., presence/absence of a nitrogen-fixing heterocyst) influence the relative abundance of cyanobacteria species, and 3) determine whether indirect, citizen-science based monitoring programs (e.g., secchi disk) adequately estimate the abundance of cyanobacteria. Specifically, because cyanobacteria are capable of fixing atmospheric nitrogen—a characteristic that sets them apart from true algae—they are at a competitive advantage when the N:P ratio is low (i.e., relatively low levels of nitrogen with relatively high levels of phosphorus). However, previous data shows Choctaw Lake to be dominated by non-nitrogen-fixing cyanobacteria, which is counterintuitive relative to these species' inability to fix nitrogen. Therefore, I hypothesize that the relative cyanobacteria abundance will increase as the N:P ratio decreases, particularly below N:P ratios of 16:1. Next, I hypothesize that when algal species compositions are dominated by cyanobacteria, heterocystous species (i.e., nitrogen-fixing) will be the most abundant. Finally, I hypothesize that turbidity, as measured by a secchi disk, will inversely correlate with direct measures of the biomass of both green algae and cyanobacteria. Together, I aim to provide citizen-science based monitoring programs with an assessment of the effectiveness of indirect measure of algal biomass in lakes (i.e., via turbidity).

Methods

Study Site

Choctaw Lake, located near London, Ohio, USA (39°57'N, 83°29'W), is a 285-acre man-made lake that was constructed in 1964. The maximum depth of the lake is 4.3 m, and the

average depth is 2.4 m. Because this is a relatively shallow lake, it does not regularly become thermally stratified. The lake has 12 kilometers of shoreline with a high degree of human development. Specifically, there are approximately 1,000 private lots, 850 of which currently have constructed homes. The lake is primarily used for recreation (e.g., boating, swimming, etc.) and does not serve as a source of drinking water. HABs were first observed in 2012 and have been occurring each year since. In 2013, water samples were analyzed and determined to contain cyanobacteria from the genera *Anabaena*, *Anabaenopsis*, *Aphanocapsa*, *Microcystis*, *Planktolyngbya*, and *Planktothrix*.

Choctaw Lake is managed by the community's Lake Water Quality Committee (LWQC), which has partnered with the Ohio Lake Management Society (OLMS) under its Citizen Lake Awareness and Monitoring program (CLAM) in order to monitor lake water quality throughout the summer season. Through this program, the LWQC collects measurements of water temperature, water color, and secchi-disk depth, for which they have data from 2012–2014. Our data collection in 2014 complements the data they have been collecting.

Nutrient Samples

We analyzed several forms of both nitrogen and phosphorus. These included total nitrogen (TN), total kjehldahl nitrogen (TKN), nitrate (NO_3^-), ammonium (NH_4^+), total phosphorus (TP), and soluble reactive phosphorus (SRP). TKN is organically-bound, so it is found in living tissue and is unavailable for algal growth. NO_3 and NH_4, on the other hand, are inorganic forms of N that are both common sources of N for algae. However, NH_4 readily adsorbs to soil particles, and so is not abundant in the water column. NO_3 is soluble, and is the most abundant source of N available for uptake. TN is calculated as the sum of TKN and NO_3. Nitrite (NO_2) was not measured as it is scarce in aquatic ecosystems due to its rapid conversion

to NO_3 by the bacteria *Nitrobacter*. SRP is largely composed of orthophosphate (PO_4), which is the form of phosphorus directly taken up by algae. However, because levels of SRP can be so low in the water column, TP—which is the sum of all soluble and particulate forms of P—is often used instead as a predictor of algal biomass. Many of the forms of P comprising TP can be oxidized into PO_4 and subsequently be assimilated by algae.

To monitor levels of nitrogen and phosphorus in the lake over the course of a field season, we collected a 1-liter water sample biweekly from four different locations in Choctaw Lake from April 2014–October 2014 (n = 4 per sampling period: Figure 1). Water samples were frozen and sent to the water quality lab of Ohio State University's Stone Laboratory. Stone Lab followed EPA standard methods 353.2, 365.1, and 350.1 for the analysis of NO_3, SRP, and NH_4 respectively (O'Dell 1993). For TP, unfiltered water was used and digested with sulfuric acid and persulfate in an autoclave, following EPA standard method 365.4. TKN was also measured with unfiltered water, and was digested with sulfuric acid and copper sulfate in a block digester, following EPA standard method 351.2. All analyses were carried out on a SEAL Analytical QuAAtro nutrient analyzer. Temperature, pH, dissolved oxygen, and conductivity were measured *in situ* at each sampling location on each sampling event. Measurements were taken every half meter to the lake bottom using a YSI 556 MPS.

To indirectly quantify phytoplankton biomass, a secchi disk and Hach turbidimeter were used to measure water clarity. Incoming sediments are considered a primary source of P inputs into many aquatic systems. To evaluate the degree of sedimentation in the lake, a water sample was taken at each of the four sampling locations during each sampling event to measure total suspended solids, volatile suspended solids, and ash free dry mass. Samples were processed

using the methods outlined by the Environmental Sciences Section of the Wisconsin State Lab of Hygiene (Wisconsin State Lab of Hygiene 1993).

Plankton Samples

To quantify the relative abundance of cyanobacteria and green algae species in the lake, 250-mL samples were collected biweekly using an 80-µm plankton net at each of the 4 sample sites within the lake. The net was continuously swept until the bottle had filled with water, and algal fragments left in the net were rinsed into the bottle with deionized (DI) water. Immediately upon returning to the lab, these samples were preserved with a 0.5% glutaraldehyde solution in order to preserve structure and color for identification and stored at 23°C. We used-1 mL subsamples to identify and count algae under a compound microscope, and used these numbers to calculate the number of cells per liter. We repeated this process for two separate samples from each of eight sampling events, and averaged the results from each. In order to quantify the relative abundance of cyanobacteria and green algae, I used a dual-channel, handheld fluorometer to measure chlorophyll-a and phycocyanin. Chorophyll-a is the dominant pigment in green algae and a standard approach for quantifying the abundance of green algae (Sartory and Grobbelaar 1984). Phycocyanin is the dominant pigment in cyanobacteria and one approach used to quantify the abundance of cyanobacteria. We also explored other indirect measures of algal biomass, including ash free dry mass. For this analysis, we filtered 200 mL of sample through glass fiber filters, dried the filters at 105°C, and placed the filters in a muffle furnace at 550°C. To measure the mass lost, we weighed the filters on a mass balance between dryings.

Statistical Analyses

Data from the four sampling locations in the lake were averaged to produce a single data point for each sampling event (i.e., sampling event represented the unit of replication, 13

sampling events, total n = 13). I used simple linear regressions to evaluate the effect of various abiotic variables on different measures of phytoplankton and cyanobacteria. In order to test how N:P ratio influences cyanobacteria biomass relative to green algae, we analyzed the effect of N:P ratio on phycocyanin:chlorophyll-a (PC:CHL-a) ratio. In calculating the N:P ratio, NO_3 was used instead of total nitrogen (TN) as NO_3 was more strongly correlated to the PC:CHL-a ratio (NO_3: $r^2 = 0.84$, $F = 47.58$, $p < 0.0001$; TN: $r^2 = 0.17$, $F = 1.84$, $p = 0.21$). This is because NO_3 is a dissolved form of nitrogen and readily available in the water column. However, we also included analyses using the TN:TP ratio in order to be consistent with calculations of the Redfield ratio (Redfield 1936). TN was calculated as the sum of NO_3 and TKN. Finally, in order to test the utility of secchi disk in monitoring the occurrence of HABs, we ran a correlation analysis analyzing the effects of PC:CHL-a ratio on secchi-disk depth.

Results

Summary of results

Tables 2–4 provide descriptive statistics for N:P ratio, PC:CHL-a ratio, and secchi-disk depth, at each sampling event. Over the course of the field season, there was a general trend whereby cyanobacteria would increase following decreased N:P ratios, and vice versa (Figure 2). In late summer, nitrogen levels approached zero, driving N:P ratios toward zero as well. In April and early May, all forms of nitrogen and phosphorus were relatively high because of increased runoff from snow melt and increased precipitation. After a sharp decline, all nutrients—except for NH_4 and NO_3—again increased and fluctuated slightly throughout the rest of the season (Figures 3 and 4).

Effects of N:P Ratio on cyanobacterial dominance

There was a significant negative relationship between N:P ratio and the relative abundance of cyanobacteria (measured as ratio of phycocyanin:chlorophyll-a, or PC:CHL-a) (r^2 = 0.91, F = 90.68, p < 0.0001: Figure 5). Though it was not as strong, a significant negative relationship was also found between TN:TP ratio and PC:CHL-a (r^2 = 0.62, F = 14.54, p = 0.0041: Figure 6). A significant positive relationship was also found between TKN and PC:CHL-a ratio (r^2 = 0.58, F = 12.65, p = 0.0062). There was no significant relationship between NH_4 and PC:CHL-a ratio (r^2 = 0.078, F = 0.76, p = 0.41). However, there was a significant positive relationship between total suspended solids (TSS) and square-root transformed NH_4 (r^2 = 0.35, F = 6.02, p = 0.03: Figure 7). Further, there was a significant positive relationship between PC:CHL-a ratio and total phosphorus (TP; r^2 = 0.75, F = 27.56, p = 0.0005), but a significant negative relationship between PC:CHL-a and NO_3 (r^2 = 0.84, F = 47.58, p < 0.0001). A summary of relationships between variables can be found in Table 5. The only genus of cyanobacteria found in the plankton sample identifications were those of *Planktothrix* (Table 6).

Indirect vs. direct measures of algal biomass

A strong significant positive correlation was found between PC:CHL-a and secchi-disk depth (r = 0.94, F = 75.48, p < 0.0001; Figure 8). No relationships were found between secchi depth and either TSS or ash free dry mass (TSS: r = 0.43, F = 2.12, p = 0.18; AFDM: r = 0.53, F = 3.67, p = 0.09). A positive correlation was, however, found between secchi depth and chlorophyll-a (r = 0.62, F = 5.48, p = 0.04) along with a negative correlation between secchi depth and phycocyanin (r = 0.79, F = 14.47, p = 0.0042). Finally, a positive correlation was found between PC:CHL-a and ash free dry mass (r = 0.64, F = 6.38, p = 0.03).

Discussion

The strong relationship found between PC:CHL-a and N:P ratio supports our first hypothesis: that the relative abundance of cyanobacteria will increase as N:P ratios decrease. Though PC:CHL-a correlated strongly with both NO_3 and TP, the N:P ratio explained significantly more of the variation in PC:CHL-a. This is likely because the N:P ratio incorporates both the amount of TP available to cyanobacteria and the amount of NO_3 limiting algal growth. However, TN:TP ratios never fell below 20:1, and therefore never reached the Redfield ratio of 16:1. This may explain why the cyanobacteria assemblage in Choctaw Lake is able to be dominated by Planktothrix, a non-heterocystous species of cyanobacteria rather than a heterocystous species, as we had originally hypothesized. Yet, it does raise questions about how *Planktothrix* is able to dominate this N-limited system when heterocystous species have occurred here previously. Finally, our results indicated that water clarity, measured by a secchi disk, is strongly correlated with more direct measures of phytoplankton and cyanobacteria biomass in Choctaw Lake.

While our results provide additional support of the relationship between N:P ratios and phytoplankton assemblages in eutrophic lakes, they also suggest that non-heterocystous species of cyanobacteria can also easily outcompete phytoplankton under low nitrogen conditions. Typically, most research has found that low N:P ratios encourage the dominance of N-fixing cyanobacteria, while non-N-fixing cyanobacteria and phytoplankton diminished in numbers (Vrede et al. 2009; Levine and Schindler 1999). Yet, our results show that the relative abundance of non-N-fixing cyanobacteria (e.g., *Planktothrix*) correlate with the N:P ratio in the same way as N-fixing cyanobacteria observed in similar studies, suggesting the non-heterocystous species are also capable of obtaining N that is unavailable to other phytoplankton. This is further supported by the negative relationship between cyanobacteria abundance and NO_3, and the positive

relationship between cyanobacteria abundance and TP. Previous studies have suggested this could be due to an ability of cyanobacteria to migrate vertically in the water column and access sedimentary sources of NH_4 (Ferber et al. 2004). The low levels of both aqueous NO_3 and NH_4 (i.e., bioavailable sources of N) might support this idea, considering there is limited N in the water column. Though this study did not investigate N levels in the substrate, this is likely a source of NH_4, which—as a cation—adsorbs strongly to soil particles. In addition, the positive correlation between total suspended solids (TSS) and NH_4 suggests that incoming sediments are bringing with them sources of NH_4, which sinks with the sediment to the substrate. Therefore, there may be a significant source of nitrogen available in lake sediments that facilitate cyanobacteria outcompeting green algae and drive harmful algal blooms.

While not all cyanobacteria are capable of exploiting sedimentary NH_4, *Planktothrix* spp. do contain the gas vacuoles necessary for efficient vertical migration, and are able to do so with greater speed than flagellated algae (Komarek 2003, Ferber et al. 2004). This could explain the persistence of *Planktothrix* despite low bioavailable N levels in the water column, which is a plausible explanation considering the shallow depth of the lake. Ferber et al. (2004) also found that, even among heterocystous cyanobacteria, ammonium was the primary source of N, with only 2% of N being supplied by N fixation. This is because the process of nitrogen fixation is energetically taxing, so it is avoided when possible (Paerl et al. 1981). Another possible explanation for the dominance of non-heterocystous cyanobacteria is that the TN:TP ratio never reached 16:1 or below, which is often noted as the ratio at which heterocystous cyanobacteria begin to dominate (Pearsall 1932, Redfield 1936, Schindler 1977). However, TN:TP ratios remained high because of elevated TKN levels, while NO_3 levels were actually near zero. TKN is organically-bound, and considered unavailable for growth. This implies N was still limiting,

despite ratios above 20:1, and that the cyanobacteria were acquiring N from sources other than the water itself.

A strong correlation was also found between cyanobacteria abundance and secchi-disk depth. This finding provides lake managers a simple model with which they can assess the abundance of cyanobacteria with relative accuracy at a very low cost. However, it is important to note the limitations of this model. The model is system-specific, and may not apply to other lake systems. This is due to different combinations of algae and sediments in the water affecting water clarity, as well as different species of algae potentially contributing different pigments, or different concentrations of pigments, that affect clarity. In addition, secchi depth does not differentiate algae; it cannot discriminate cyanobacteria from green algae from diatoms, etc. However, for a system such as Choctaw Lake where it is known that the lake is plagued by cyanobacteria and which species of cyanobacteria it is plagued by, developing such a model can be an effective tool for monitoring their water quality without investing in more expensive analytical instruments. If resources are available to measure phycocyanin, though, other studies have shown that secchi-disk depth and phycocyanin measurements can be used to construct a multivariable logistic regression model that can predict the odds that water samples will exceed 4 µg/L of microcystin-LR (Marion et al. 2012). This can provide a quick way to assess the level of toxins in the water while keeping monitoring costs relatively low.

These findings prove useful for management purposes. While further studies will be needed to confirm high NH_4 concentrations in the substrate, the correlation between TSS and the NH_4 provides enough evidence to warrant the control of sediment inputs. Perhaps more importantly, P also adsorbs strongly to soil particles, so incoming sediments also serve as a significant source of P in aquatic systems. Therefore, sediments are contributing two nutrients

that are likely key for the lake's dominant cyanobacteria—NH_4 and P. In systems dominated by heterocystous cyanobacteria, P is a limiting nutrient because they have an effectively infinite source of N (i.e., atmospheric N, N_2). However, in systems such as Choctaw Lake, which are dominated by non-heterocystous cyanobacteria, it is possible that both NH_4 and P are limiting nutrients. Further research will be needed to clarify.

Limitations

The primary limitation to this study is its small sample size and restriction to one field season of data collection. This limits our ability to observe longer term dynamics with cyanobacteria blooms which may help to address some of the uncertainties about why *Planktothrix* is dominating in this system. Likewise, this study assessed at these dynamics in only one lake. This restricts our ability to generalize our findings, so it would have been useful to conduct similar sample collections among a variety of lakes of similar size and depth. Though our model can be a good predictor of cyanobacteria biomass, it does not reveal whether or not the cyanobacteria are actually producing toxins. It might, however, show the potential for toxin production. Further, collecting sediment samples for nutrient concentrations in the substrate would have provided a more comprehensive understanding of how nutrients were being acquired by these cyanobacteria. However, these limitations provide avenues for further research in this system.

Literature Cited

Barica J, Kling H, Gibson J (1980) Experimental manipulation of algal bloom composition by nitrogen addition. *Canadian Journal of Fisheries and Aquatic Sciences* 37:1175-1183

Carmichael WW, Jones CLA, Mahmood NA, Theiss WC (1985) Algal toxins and water-based diseases. *CRC Critical Review of Environmental Control* 15:275-313

Carpenter SR, Caraco NF, Correll DL, Howarth RW, Sharpley AN, Smith VH (1998) Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications* 8:559-568

Codd GA, Poon GK (1988) Biochemistry of the Algae and Cyanobacteria. Clarendon Press, Oxford

Codd GA, Bell SG, Brooks WP (1989) Cyanobacterial toxins in water. *Water Science and Technology* 24:1-13

Codd GA, Bell SG, Kaya K, Ward CJ, Beattie KA, Metcalf JS (1999) Cyanobacterial toxins, exposure routes and human health. *European Journal of Phycology* 34:405-415

Falconer IR (1989) Effects on human health of some toxic cyanobacteria (blue-green algae) in reservoirs, lakes, and rivers. *Environmental Toxicology and Water Quality* 4:175-184

Ferber LR, Levine SN, Lini A, Livingston GP (2004) Do cyanobacteria dominate in eutrophic lakes because they fix atmospheric nitrogen? *Freshwater Biology* 49:690-708

Havens KE, James RT, East TL, Smith VH (2003) N:P ratios, light limitation, and cyanobacterial dominance in a subtropical lake impacted by non-point source nutrient pollution. *Environmental Pollution* 122:379-390

Hillerbrand H, Bennett DM, Cadotte MW (2008) Consequences of dominance: A review of evenness effects on local and regional ecosystem processes. *Ecology* 89:1510-1520

Huber AL, Hamel KS (1985) Phosphatase activities in relation to phosphorus nutrition in *Nodularia spumigena* (Cyanobacteriaceae). *Hydrobiologia* 123:145-152

Jochimsen EM, Wayne MD, Carmichael W, An J, Cardo DM, Cookson ST, Holmes CEM, Antunes MB, de Melo Filho DA, Lyra TM, Barreto VS, Azevedo SM, Jarvis WR (1998)

Liver failure and death after exposure to microcystin at a hemodialysis center in Brazil. *The New England Journal of Medicine* 338:873-878

Kahru M, Leppanen JM, Rud O, Savchuk OP (2000) Cyanobacteria blooms in the Gulf of Finland triggered by saltwater inflow into the Baltic Sea. *Marine Ecology* 207:13-18

Komarek J (2003) Planktic oscillatorialean cyanoprokaryotes (short review according to combined phenotype and molecular aspects). *Hydrobiologia* 502:367-382

Levin SN, Schindler DW (1999) Influence of nitrogen to phosphorus supply ratios and physicochemical conditions on cyanobacteria and phytoplankton species composition in the Experimental Lakes Area, Canada. *Canadian Journal of Fisheries and Aquatic Sciences* 56:451-466

MacKintosh C, Beattie KA, Klumpp S, Cohen P, Codd GA (1990) Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphates 1 and 2A from both mammals and higher plants. *Federation of European Biochemical Societies* 264:187-192

Marion JW, Lee J, Wilkins JR, Lemeshow S, Lee C, Waletzko EJ, Buckley TJ (2012) In vivo phycocyanin flourometry as a potential rapid screening tool for predicting elevated microcystin concentrations at eutrophic lakes. *Environmental Science & Technology* 46:4523-4531

Mulholland MR, Floge S, Carpenter EJ, Carpone DG (2002) Phosphorus dynamics in cultures and natural populations of *Trichodesmium spp. Marine Ecology*. 239:45-55

Nurnberg GK, Shaw M (1999) Productivity of clear and humic lakes: nutrients, phytoplankton, bacteria. *Hydrobiologia* 382:97-112

O'Dell J (1993) Method 350.1 Determination of ammonia nitrogen by semi-automated colorimetry. Environmental Monitoring Systems Laboratory. U.S. Environmental Protection Agency.

O'Dell J (1993) Method 353.2 Determination of nitrate-nitrite nitrogen by automated colorimetry. Environmental Monitoring Systems Laboratory. U.S. Environmental Protection Agency.

O'Dell J (1993) Method 365.1 Determination of phosphorus by semi-automated colorimetry. Environmental Monitoring Systems Laboratory. U.S. Environmental Protection Agency.

Osborne LL, Kovacic DA (1993) Riparian vegetated buffer strips in water-quality restoration and stream management. *Freshwater Biology* 29:243-258

Paerl HW, Webb KL, Baker J, Weibe W (1981) Nitrogen fixation in water. *Nitrogen Fixation* 193-240

Pinto P, Litchman E (2010) Interactive effects of N:P ratios and light on nitrogen-fixer abundance. *Oikos* 119:567-575

Pearsall WH (1932) Phytoplankton in the English Lakes I. The composition of the phytoplankton in relation to the dissolved substances. *Journal of Ecology* 20:241-262

Redfield AC (1936) An Ecological Aspect of the Gulf Stream. *Nature* 138:1013

Rouhilainen L, Sivonen K, Buikema WJ, Haselkorn R (1995) Characterization of toxin-producing cyanobacteria by using an oligonucleotide probe containing a tandemly repeated heptamer. *Journal of Bacteriology* 177:6021-6026

Sartory DP, Grobbelaar JU (1984) Extraction of chlorophyll *a* from freshwater phytoplankton for spectrophotometric analysis. *Hydrobiologia* 114:177-187

Schindler DW (1977) Evolution of phosphorus limitation in lakes. *Science* 46:260-262

Sellner KG, Doucette GJ, Kirkpatrick GJ (2003) Harmful algal blooms: causes, impacts and detection. *Microbiological Biotechnology* 30:383-406

Smith VH (1983) Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. *Science* 221:669-671

Stockner JF, Shortweed KS (1988) Response of *Anabaena* and *Synechococcus* to manipulation of nitrogen: phosphorus ratios in a lake fertilization experiment. *Limnology and Oceanography* 33:1348-1361

Trimbee AM, Prepas EE (1988) The effect of oxygen depletion on the timing and magnitude of blue-green algal blooms. *Limnology* 23:220-226

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG (1997) Human alteration of the global nitrogen cycle: Sources and consequences. *Ecological Applications* 7:737-750

Vrede T, Ballantyne A, Mille-Lindblom M, Algesten G, Gudasz C, Lindahl S, Brunerg AK (2009) Effects of N:P loading ratios on phytoplankton community composition, primary production and N fixation in a eutrophic lake. *Freshwater Biology* 54:331-344

Webster KE, Peters Rh (1978) Some size-dependent inhibitions of larger cladoceran filterers in filamentous suspensions. *Limnology and Oceanography* 23:1238-1245

Wisconsin State Lab of Hygiene (1993) ESS Method 340.2: Suspended solids, mass balance. Environmental Sciences Section. Inorganic Chemistry Unit.

Appendix

Table 1. Independent and dependent variables studied.

Independent Variables	**Dependent Variables**
Temperature (°C)	Chlorophyll-a (ppb)
Dissolved oxygen (DO) (mg/L)	Phycocyanin (ppb)

Conductivity (mS/cm)	Total suspended solids (mg/L)
Total nitrogen (TN) (µmol/L)	Ash free dry mass (mg/L)
Total kjehldahl nitrogen(TKN) (µmol/L)	
Nitrate (NO_3) (µmol/L)	
Ammonium (NH_4) (µmol/L)	
Total phophorus (TP) (µmol/L)	
Soluble Reactive Phosphorus (SRP) (µmol/L)	
N:P Ratio	
Secchi disk depth (cm)	
Turbidity (NTU)	

Table 2. Descriptive statistics of N:P ratio from April-October 2014.

N:P Ratio	*4/30*	*5/13*	*5/27*	*6/11*	*6/30*	*7/16*	*7/31*	*8/14*	*8/25*	*9/5*	*9/25*	*10/16*	*10/30*
Mean	28.126	19.304	29.371	19.646	28.834	10.353	0.066	0.095	0.028	0.008	1.417	0.052	0.030
Standard Error	2.767	3.568	4.428	1.189	0.875	1.310	0.018	0.029	0.019	0.006	1.417	0.017	0.005
Standard Deviation	5.535	7.136	8.857	2.377	1.750	2.620	0.036	0.059	0.039	0.013	2.834	0.035	0.009
Sample Variance	30.633	50.928	78.441	5.652	3.062	6.862	0.001	0.003	0.001	0.000	8.033	0.001	0.000
Minimum	22.199	12.264	20.897	17.256	26.366	8.138	0.040	0.039	0.000	0.000	0.000	0.000	0.020
Maximum	33.097	26.040	37.963	22.384	30.379	13.397	0.116	0.157	0.082	0.027	5.668	0.074	0.043
Count	4	4	4	4	4	4	4	4	4	4	4	4	4

Table 3. Descriptive statistics of phycocyanin:chlorophyll-a (PC:CHL-a) ratio from May-October 2014.

PC:CHL-a Ratio	*5/27*	*6/11*	*6/30*	*7/16*	*7/31*	*8/14*	*8/25*	*9/5*	*9/25*	*10/16*	*10/30*
Mean	4.477	21.683	14.034	26.342	41.966	48.167	46.109	43.330	37.921	46.711	35.678
Standard Error	0.553	0.721	1.637	2.334	5.070	5.352	3.145	6.484	4.941	1.936	1.052
Standard Deviation	1.106	1.443	3.274	4.668	10.140	10.704	6.290	12.969	9.881	3.873	2.105
Sample Variance	1.224	2.082	10.717	21.791	102.828	114.573	39.567	168.190	97.642	14.998	4.430
Minimum	3.772	20.847	9.490	20.278	32.010	38.035	40.610	24.748	23.166	41.771	33.134
Maximum	6.100	23.832	17.304	31.492	52.196	62.817	52.406	54.927	44.173	51.117	37.489
Count	4	4	4	4	4	4	4	4	4	4	4

Table 4. Descriptive statistics of secchi disk depth from May-October 2014.

Secchi Disk Depth	*5/27*	*6/11*	*6/30*	*7/16*	*7/31*	*8/14*	*8/25*	*9/5*	*9/25*	*10/16*	*10/30*
Mean	20.000	16.250	16.875	13.375	12.375	11.250	12.250	12.250	11.875	12.750	13.250
Standard Error	1.541	0.968	0.826	0.473	0.800	0.323	0.250	0.722	0.427	0.520	0.144
Standard Deviation	3.082	1.936	1.652	0.946	1.601	0.645	0.500	1.443	0.854	1.041	0.289
Sample Variance	9.500	3.750	2.729	0.896	2.563	0.417	0.250	2.083	0.729	1.083	0.083
Minimum	16.000	13.500	15.000	12.000	10.000	10.500	12.000	11.000	11.000	11.500	13.000
Maximum	23.500	18.000	18.500	14.000	13.500	12.000	13.000	13.500	13.000	14.000	13.500
Count	4	4	4	4	4	4	4	4	4	4	4

Table 5. Summary table of regressions performed in analyses.

Regression	r^2	F	p-value
N:P vs. PC:CHL-a	0.91	90.68	<0.0001
TKN vs. PC:CHL-a	0.58	12.65	0.0062
NH_4 vs. PC:CHL-a	0.078	0.76	0.41
TP vs. PC:CHL-a	0.75	27.56	0.0005
NO_3 vs. PC:CHL-a	0.84	47.58	<0.0001
PC:CHL-a vs. secchi disk depth	0.89	75.48	<0.0001
TSS vs. cell count	0.53	6.7	0.0413
TSS vs. sqrt-NH_4	0.35	6.02	0.03

Table 6. Results from cyanobacteria identification. The only species present was *Planktothrix.*

Sample Date	Cyanobacteria	Cells per Liter (x1000)
7/16/2014	*Planktothrix*	1193
7/31/2014	*Planktothrix*	215
8/14/2014	*Planktothrix*	390
8/25/2014	*Planktothrix*	194
9/5/2014	*Planktothrix*	1730
9/25/2014	*Planktothrix*	2399
10/16/2014	*Planktothrix*	2034
10/30/2014	*Planktothrix*	1905

Figure 1. Choctaw Lake, with each of the four sampling points marked with a yellow pin.

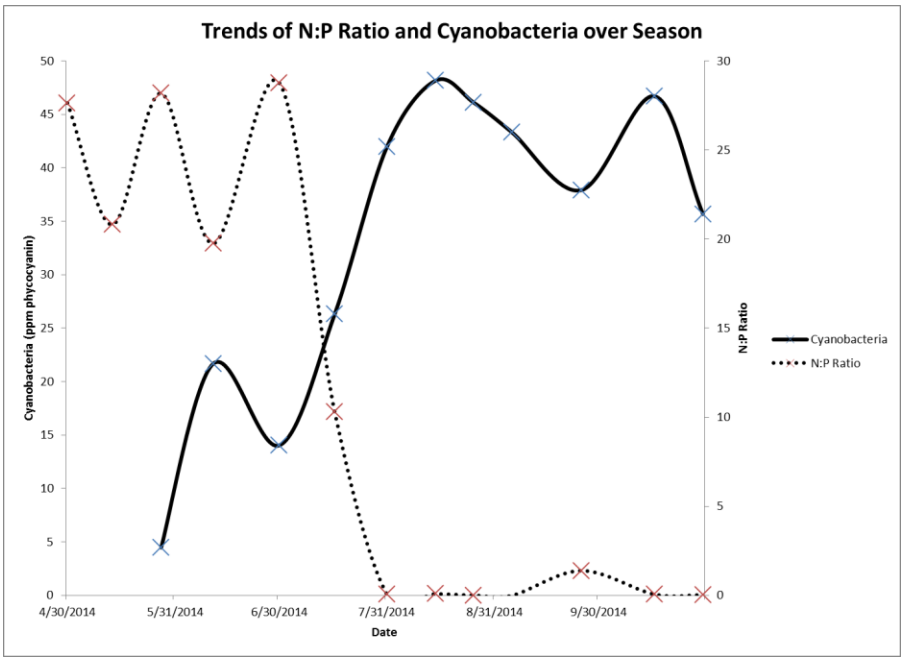

Figure 2. Trends of N:P ratio and cyanobacteria (phycocyanin) levels from April-October 2014. In general, cyanobacteria levels increase as N:P ratio decreases, and vice versa.

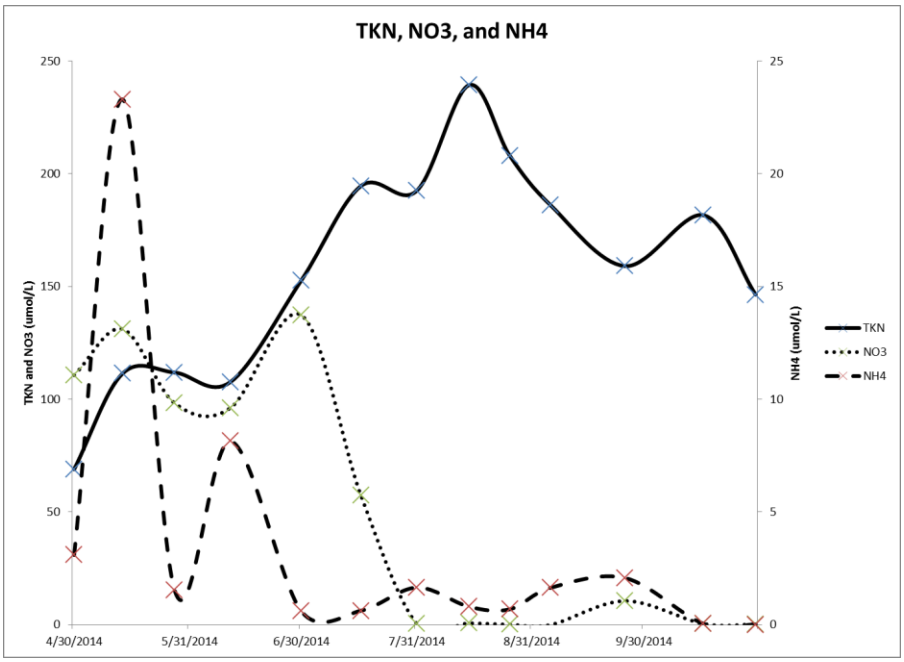

Figure 3. Dynamics of various N constituents (i.e. TKN, NO_3, and NH_4) from April-October 2014. Both NO_3 and NH_4 decrease substantially toward the end of the season. Organically-bound N (TKN) likely remains high due to high cyanobacteria levels.

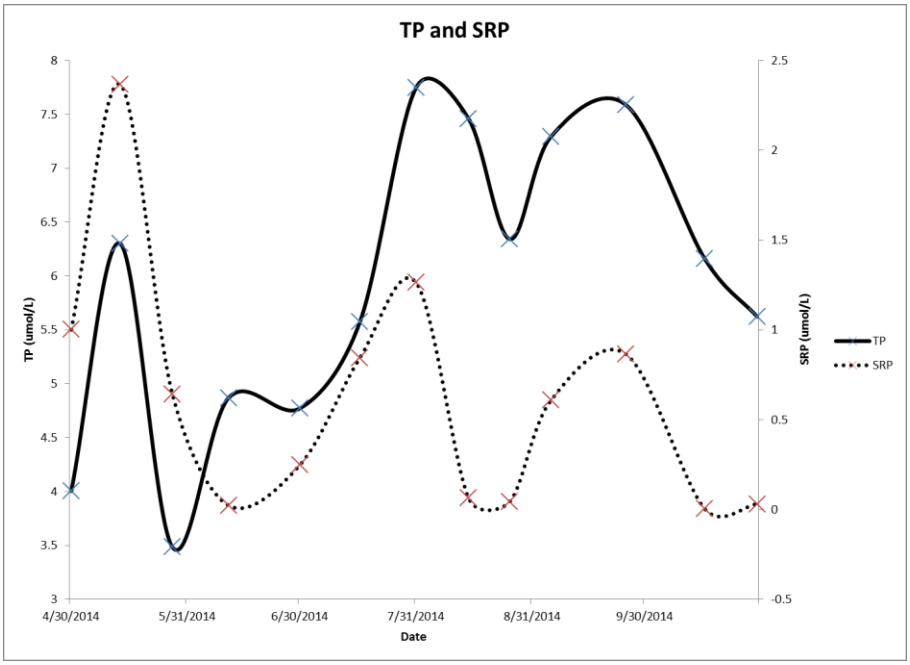

Figure 4. Dynamics of TP and SRP from April-October 2014. Levels fluctuate throughout the season.

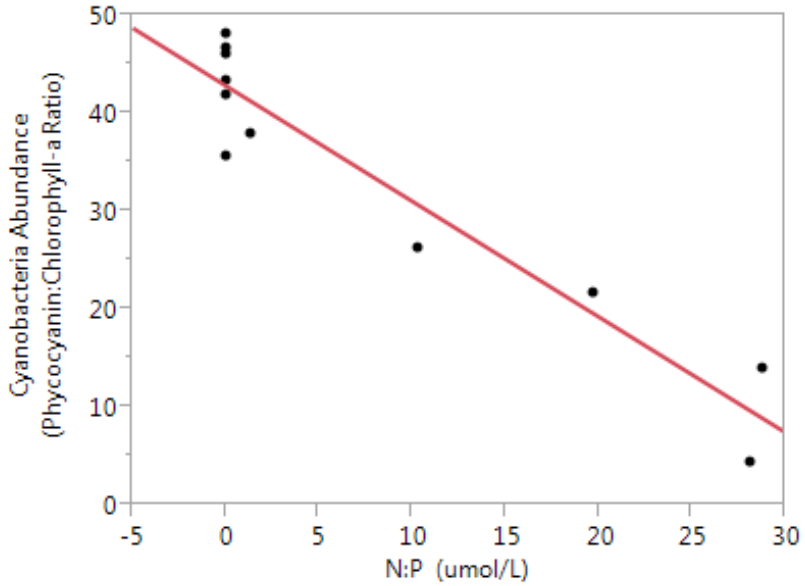

Figure 5. Correlation between N:P ratio (umol/L) and cyanobacteria abundance, measured as the ratio of PC:CHL-a. There was a strongly negative relationship between these parameters (r^2 = 0.91, F = 90.68, $p < 0.0001$).

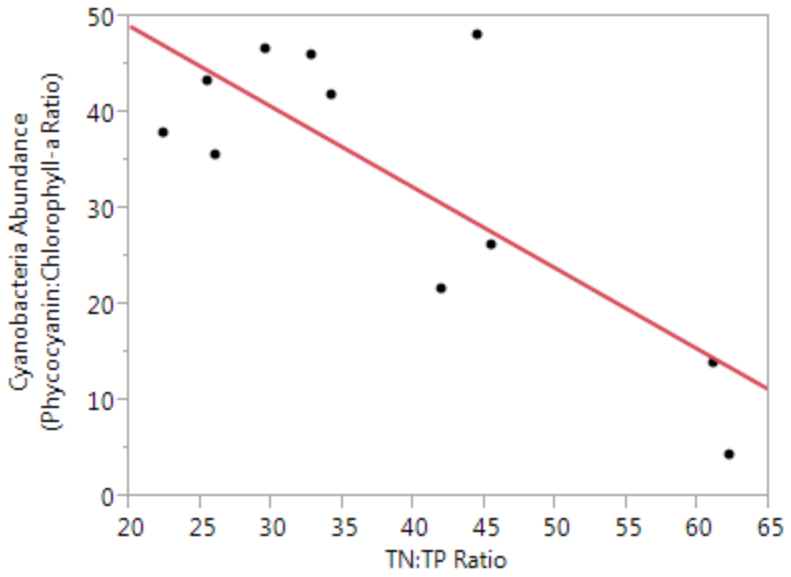

Figure 6. Relationship between TN:TP ratio and cyanobacteria abundance, measured as the ratio of PC:CHL-a. Here, there is also a negative relationship (r^2 = 0.62, F = 14.54, p = 0.0041).

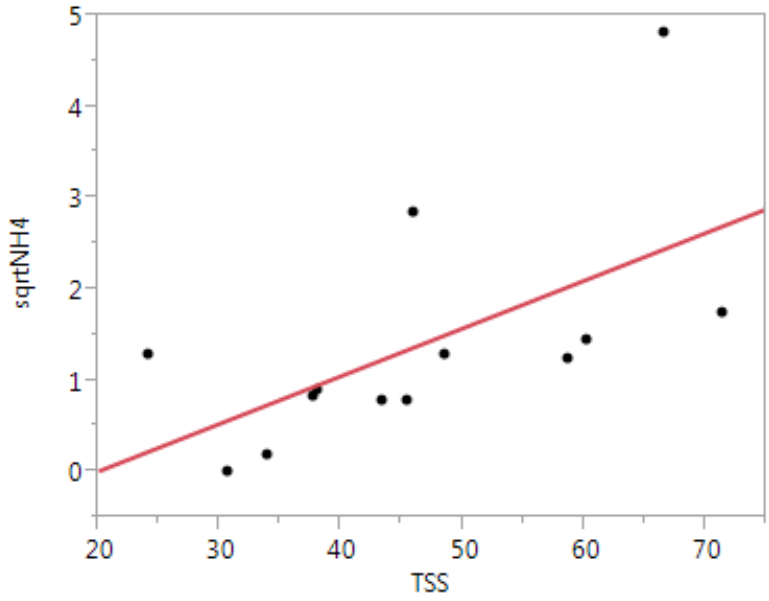

Figure 7. There is a positive correlation between total suspended solids (TSS) and the square-root transformed NH_4 (r^2 = 0.35, F = 6.02, p = 0.03).

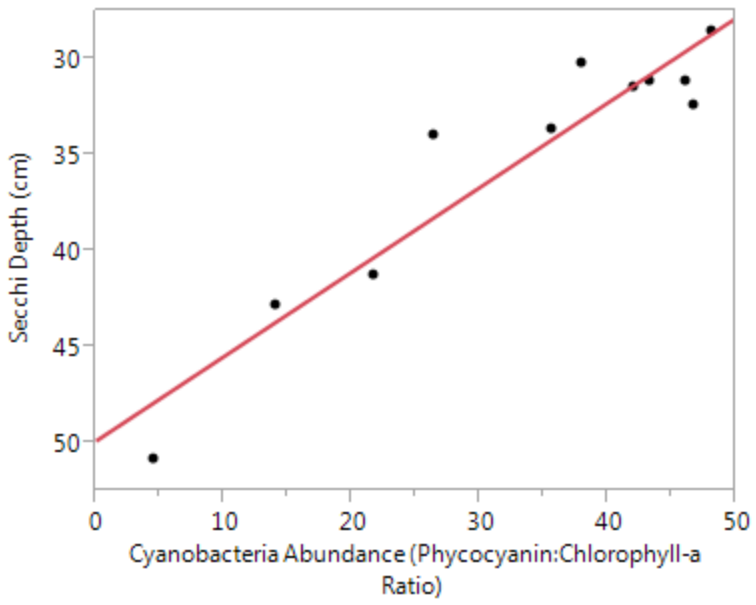

Figure 8. There is a strong positive relationship between cyanobacteria abundance and inverse secchi disk depth ($r^2 = 0.89$, $F = 75.48$, $p < 0.0001$).